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Microbial Assessment and Antibiotic Resistance of Some Microbial Isolates from Otamiri River in Owerri Metropolis, Eastern Nigeria

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Abstract:- Selected bacteria recovered from the Otamiri River were examined for antibiotic resistance using a conventional microbiological method. Four (4) sediment samples and a total of twelve (12) surface water samples were taken. The following bacteria were detected as a result of the molecular identification of microorganisms of interest: *Proteus miriabilis, Lysinibacillus macroides, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Alcaligenes faecalis*. A higher proportion of the microorganisms exhibited complete resistance (100%) to the tested drugs. The maximum percentage of susceptibility of the organisms to the test antibiotics was just 50%. Protecting natural bodies of water from environmental pollutants that contaminate these natural reservoirs is necessary due to rising antibiotic resistance among bacterial isolates and ongoing outbreaks of water-borne illnesses. Since water is the most essential necessity for both humans and animals, the current government and pertinent state authorities should enact rules and controls that forbid polluting of this water source.

I. INTRODUCTION

The availability of clean drinking water is a top worry for individuals who live in underdeveloped nations all over the world. The fact that many of the available water sources cannot be used as drinking water without being treated further complicates this issue [1]. Many places in the impoverished world lack such treatment facilities or do not have them operating. Wells, springs, streams, rivers, and lakes consequently serve as primary and direct sources of water supply for drinking and other domestic needs in the majority of rural settlements, particularly in Nigeria [2]. Several incidences of gastrointestinal illnesses like cholera, typhoid, and paratyphoid fever have been linked to the use of untreated subterranean and surface water for drinking and domestic reasons [2]. Numerous studies have suggested that fecal contamination of food and water sources is the root cause of all enteric bacterial infections. The most common symptoms of exposure to disease-causing bacteria from drinking polluted water are nausea, vomiting, and diarrhea. The most vulnerable populations to waterborne illnesses are infants and elderly individuals with compromised immune systems [3]. The difficulty caused by the ineffectiveness and failures of antimicrobial chemotherapies used in clinical practice to treat such diseases has added to the burden of disease. Antibiotic resistance is a widespread clinical and public health issue. The World Health Organization (WHO), the European Commission, and the Centers for Disease Control and Prevention (CDC) in the United States have all acknowledged the significance of researching the causes and dynamics of resistance [4]. Over the years, antibiotics have been utilized to treat illnesses in both humans and animals. However, because of the body's inadequate metabolic process, they are constantly present in the surroundings. The role of man and his numerous anthropogenic actions in the propagation of resistance components in microorganisms is not yet obvious, and disagreements among scientists concerning this topic grow every day. numerous people believe that the factors that select for resistance are naturally present within microbial genomes, and numerous studies have indicated that there is no clear association between anthropogenic activity and antibiotic resistance in bacteria [5, 6, 7]. A global phenomenon is the concern for the ecological fate and environmental threat of these pharmaceuticals in the aquatic milieu, in addition to the threats to human health posed by the environmental presence of bacteria that are resistant to antibiotics and the unintended presence of antibiotics in water bodies [8].

Antibiotic-resistant bacteria have been viewed as a threat to global public health. Antibiotic resistant bacteria (ARB) of various types are regularly found in both aquatic and terrestrial habitats. The likelihood of ARB spreading resistance from the environment to associated human pathogenic bacteria by a variety of mechanisms is significant, which would reduce the effectiveness of antibiotics [9]. Polluted water bodies represent a favorable habitat for both the survival of ARB and the transfer of antibiotic resistance due to the high microbial biomass, abundance of nutrients, as well as a variety of antimicrobial agents, from which they spread resistant bacteria into subsequent aquatic and terrestrial environments [10]. A lot of water systems had previously contained various ARB, including many antibiotic-resistant bacteria [11]. Pathogens have also been reported to damage or harm more kilometers of water bodies including rivers, streams, and lakes than any other aquatic contaminant. Microbial and sediment pollution have been shown to be serious concerns for rivers and streams. Similar to the previous point, bacterial water contamination can lead to contaminated drinking water, reduced recreational options, and closure of shellfish beds [12]. This study's objective is to assess the antibiotic resistance of a few bacteria that were isolated from the Otamiri River in Owerri, Imo State.

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II. RESOURCES AND APPROACH

A. Study Area

There are numerous natural sources of water in Owerri, the capital of Imo State. The large Otamiri River in Owerri is the subject of this investigation. The river provides a significant portion of the water needed for both home and industrial uses. Before flowing into the Atlantic Ocean, the river passes through various communities in the Owerri Local Government Areas and other rivers beyond Imo State. The river's source is Otamiri in Egbu. As all of the state's principal drainage is directed into the river, it has accumulated a ton of solid and liquid garbage over the years.

B. Water Samples

Surface water samples were taken at the Egbu slaughterhouse, Mechanic village, Nekede Bridge, and Aba Road Bridge. These samples were taken upstream, midstream, and downstream. Using a wide mouth, sterile one-liter sampling container held at an elbow depth of roughly thirty (30) centimeters, a total of twelve (12) surface water samples were taken. Grab samplers were used to collect sediment samples, one from each sampling station for a total of four (4) samples. The table below shows the coordinates of the sample collection points. Prior to analysis, samples were transported to the lab in ice block boxes.

Location	Sample ID	Latitude	Longitude
Egbu abattoir	WS 1	5.468454	7.0579694
Mechanic village	WS 2	5.454669	7.0319396
Aba road	WS 3	5.466485	7.0412746
Nekede	WS 4	5.398004	6.9934721

C. Bacterial Isolation

➢ *Proteus* and *Klebsiella* Isolation

The serially diluted sample was diluted appropriately, and 0.1 ml of that dilution was used to inoculate freshly made MacConkey agar plates. A glass rod that was bent into an L shape was used to disseminate the inoculums. They were kept in an incubator for 24 hours at 37^0C. Using the organism of interest's unique colonial morphology, colonies were selected; sub cultured into sterile nutrient agar plates, and incubated at the proper temperature. Once their purity had been established, isolates were retained by sub culturing into nutrient agar slants and stored at 4°C until needed for molecular identification studies.

➢ *Isolation of Pseudomonas*

Freshly made *Pseudomonas* centrimide agar plates were innoculated with 0.1 ml of the proper dilution of the serially diluted sample. A glass rod that was bent into an L shape was used to disseminate the inoculums. They were kept in an incubator for 24 hours at 37^0C. Using the organism of interest's unique colonial morphology, colonies were selected; sub cultured into sterile nutrient agar plates, and incubated at the proper temperature. Once their purity had been established, isolates were retained by sub culturing into nutrient agar slants and stored at 4°C for further studies.

➢ *Alcaligenes Isolation*

The serially diluted sample was diluted appropriately, and 0.1 ml of that dilution was used to inoculate on freshly made skim milk agar plates. A glass rod that was bent into an L shape was used to disseminate the inoculums. They were kept in an incubator for 24 hours at 370C. Using the organism of interest's unique colonial morphology, colonies were selected; sub cultured into sterile nutrient agar plates, and incubated at the proper temperature. When purity was established, isolates were retained by sub culturing into nutrient agar slants and stored in the refrigerator at 4^0C until needed.

➢ *Isolation of Lysinibacillus*

Freshly made nutrient agar plates were inoculated with 0.1 ml of the appropriate dilution of the serially diluted sample. A glass rod that was bent into a L shape was used to disseminate the inoculums. They were kept in an incubator for 24 hours at 37^0C. Using the organism of interest's unique colonial morphology, colonies were selected; sub cultured into sterile nutrient agar plates, and incubated at the proper temperature. When purity was established, isolates were retained by sub culturing into nutrient agar slants and stored in the refrigerator at 4^0C until needed.

➢ *Molecular Identification*

- NA extraction (Boiling technique)

The bacterial isolate's overnight broth culture in Luria Bertani (LB) was spun at 14000 rpm for three minutes using five milliliters. The cells were heated at 95^0C for 20 min before being re-suspended in 500µl of normal saline. The heated bacterial suspension was spun for three minutes at 14000 rpm after cooling on ice. For use in additional downstream procedures, the DNA-containing supernatant was transferred to a 1.5 ml micro centrifuge tube and kept at -20^0C.

- DNA quantification

The Nanodrop 1000 spectrophotometer was used to measure the amount of genomic DNA that was extracted. Double clicking on the Nanodrop icon launched the equipment's software. The apparatus was blanked with normal saline and initiated with 2µl of sterile, distilled water. The top pedestal was lowered to make contact with the extracted DNA on the lower pedestal after two microliters of the extracted DNA were put onto the lower pedestal. The "measure" button was used to calculate the DNA concentration.

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- 6S rRNA Amplification

Using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3 and 1492R: 5'-CGGTTACCTTGTTACGACTT-3 primers on an ABI 9700 Applied Biosystems thermal cycler for 35 cycles at a final volume of 40 microliters, the area of the 16s rRNA of the isolates was amplified. The X2 Dream Taq Master mix (Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers of 0.5uM, and the template, which is an extracted DNA as template, make up the PCR mix. The following temperatures and times were used during the PCR process: initial denaturation for 5 minutes at 95°C; denaturation for 30 seconds at 95^0C; annealing for 30 seconds at 52^0C; extension for 35 cycles at 72°C; and final extension for 5 minutes at 72°C. The result was seen on a blue light trans illuminator after being resolved on a 1% agarose gel at 130V for 30 minutes.

- Squencing

Inqaba Biotechnological, Pretoria, South Africa, was used to perform the sequencing using a 3510 ABI sequencer and the BigDye Terminator kit. The BigDye® v1.1/v3.1, 0.25µl of the 10µM Primer PCR primer, 2.25µl of the 5 x BigDye sequencing buffer and 2-10ng of PCR template per 100bp were used in the sequencing which was done at a final volume of 10µl. The next 32 cycle of 96^0C for 10s, and 60^0C for 4min were the sequencing conditions.

- Phylogenetic Analysis

The bioinformatics algorithm Trace edit was used to alter the obtained sequences, and BLASTN was used to retrieve related sequences from the National Center for Biotechnology Information (NCBI) database. Using MAFFT, these sequences were aligned. With MEGA 6.0's Neighbor-Joining approach, the evolutionary history was deduced [13]. The evolutionary history of the taxa under study is assumed to be represented by the bootstrap consensus tree generated from 500 replicates [14]. The Jukes-Cantor technique was used to calculate the evolutionary distances [15].

D. Standardization of inoculum

Using 0.5 Macfarland standard solutions, the inoculum for antibacterial susceptibility tests were standardized to an inoculum density of 1.0 x 10^8 cfu/ml. In a nutshell, sterile wire loops were used to pull out 4 to 5 distinct colonies from nutrient agar plates, and they were then emulsified into tubes containing normal saline, 0.9% w/v. A turbid suspension was created by thoroughly mixing the tubes using a vortex. The turbidity and Macfarland standard solution marched in good lighting.

E. Antibacterial Susceptibility test

The method outlined by [15] was adopted to conduct the antimicrobial susceptibility test. Briefly, Muller Hinton agar plates were filled with 0.1 ml of the standardized bacterial inoculum. With a clean L-shaped glass rod, the inoculant was dispersed. In order for the media to absorb into the inoculum, the plates were permitted to stand on the laboratory bench for 20 minutes. Using sterile forceps, commercial antibiotics disc from the United Kingdom's Oxoid was applied to the inoculated plates. The plates were placed inverted and incubated at 37^0C for 18 hours. Using a ruler and millimetre (mm) units, the diameter of developing zones of inhibition was measured, reported, and analyse [16].

III. RESULTS

A. Bacteria isolates

The results of the molecular identification of the pure culture of bacteria isolates revealed that the following bacteria were identified viz: *Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Alcaligenes faecalis, Lysinibacillus macroides.*

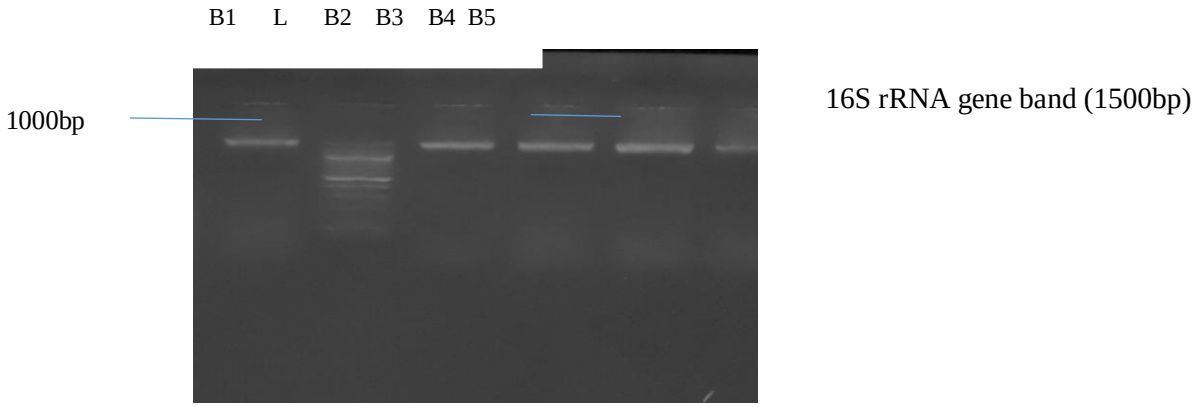

Plate 1: Agarose gel electrophoresis of the 16S rRNA gene of bacterial isolates. Lanes B1-B5 represent the 16SrRNA gene bands (1500bp), lane L represents the 100bp molecular ladder.

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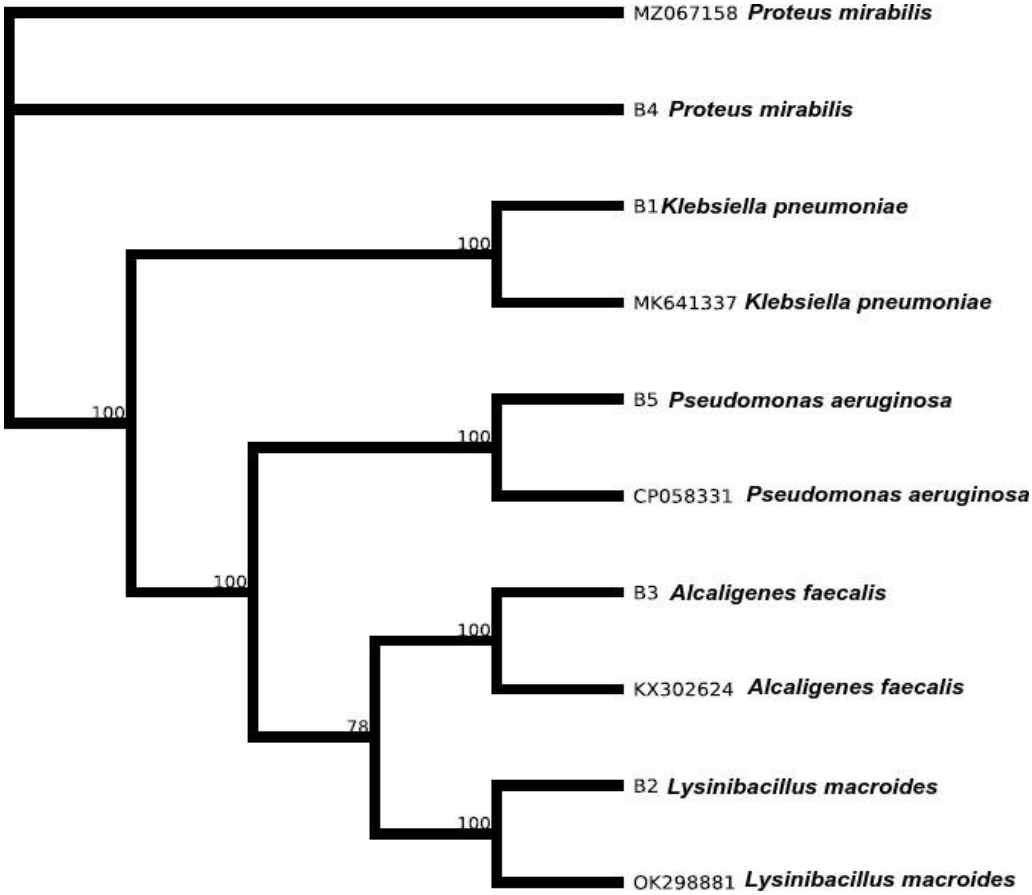

Fig. 1: Phylogenic tree of molecular identified bacteria isolates

B. Percentage of antibiotics susceptibility test on pure culture of bacteria isolates

Alcaligenes faecalis is the organism that is most susceptible to the medications tested, according to the results of the antibiotics susceptibility test shown in Figure 1. It demonstrated susceptibility to seven out of the ten drugs tested, being susceptible on seven occasions on PEF, CPX, GN, CEP, SXT, STR, PN and NA, followed by *Proteus miriabilis* showing susceptibility on OFX, AMC, PEF, CPX and SXT. However *Pseudomonas aeruginosa* and *Lysinibacillus macroides*, had the least susceptibility among the antibiotics tested.

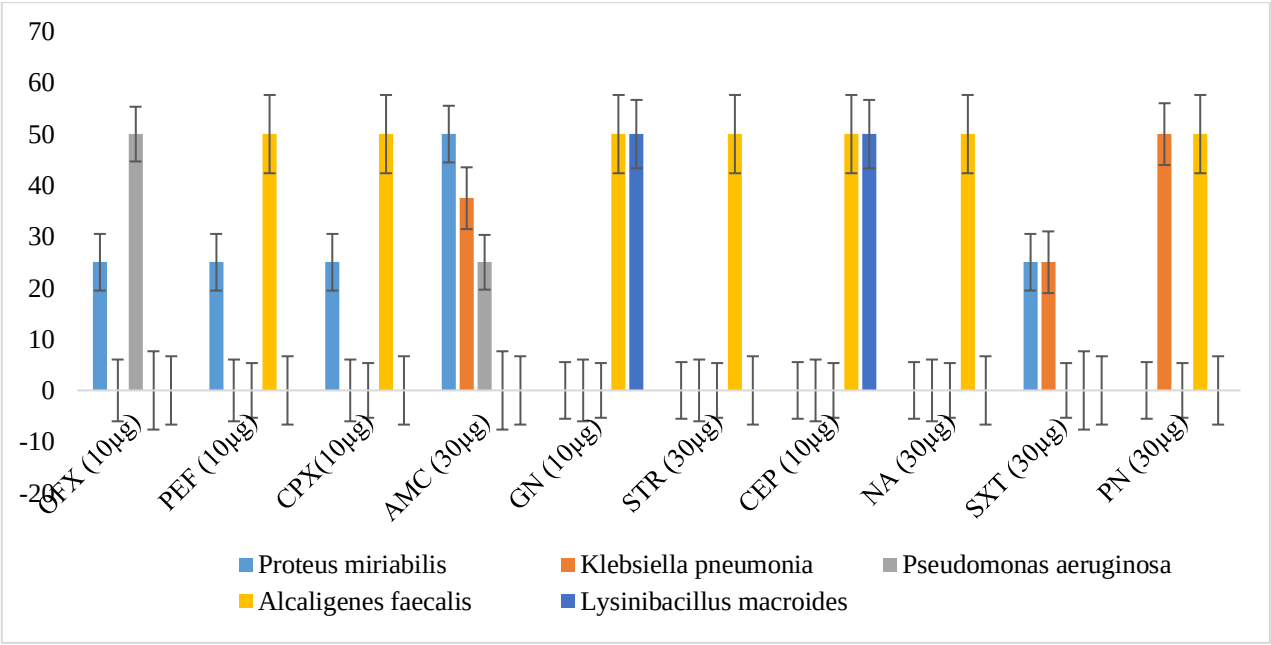

Fig. 2: Percentage of antibiotics susceptibility of selected bacteria isolates
PEF= pefloxacin, GN= gentamycin, AMC = amoxacillin clauvanic acid, CPX= ciprofloxacin, STR= streptomycin, SXT= septrin, OFX= ofloxacin, CEP = ciprorex, NA= nalidixic acid, PN = ampicillin

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C. Percentage of antibiotics resistant test on pure culture of bacteria isolates

Pseudomonas aeruginosa and *Lysinibacillus macroides* were found to be the bacteria isolates with the highest antibiotic resistance, as evidenced by their resistance to eight out of the ten antibiotics tested, as shown in figure 2. The following medications were resistant to *Pseudonomas aeruginosa*: PEF, CPX, GN, STR, CEP, NA, SXT and PN while *Lysinibacillus macroides* showed resistance to OFX, AMC, PEF, CPX, STR, NA, SXT and PN. *Klebsiella pneumonia* showed resistance to seven (7) antibiotics, *Proteus miriabilis* to five (5) antibiotics while *Alcaligenes faecalis* had the least resistance showing that to two (2) antibiotics out of the ten (10) tested.

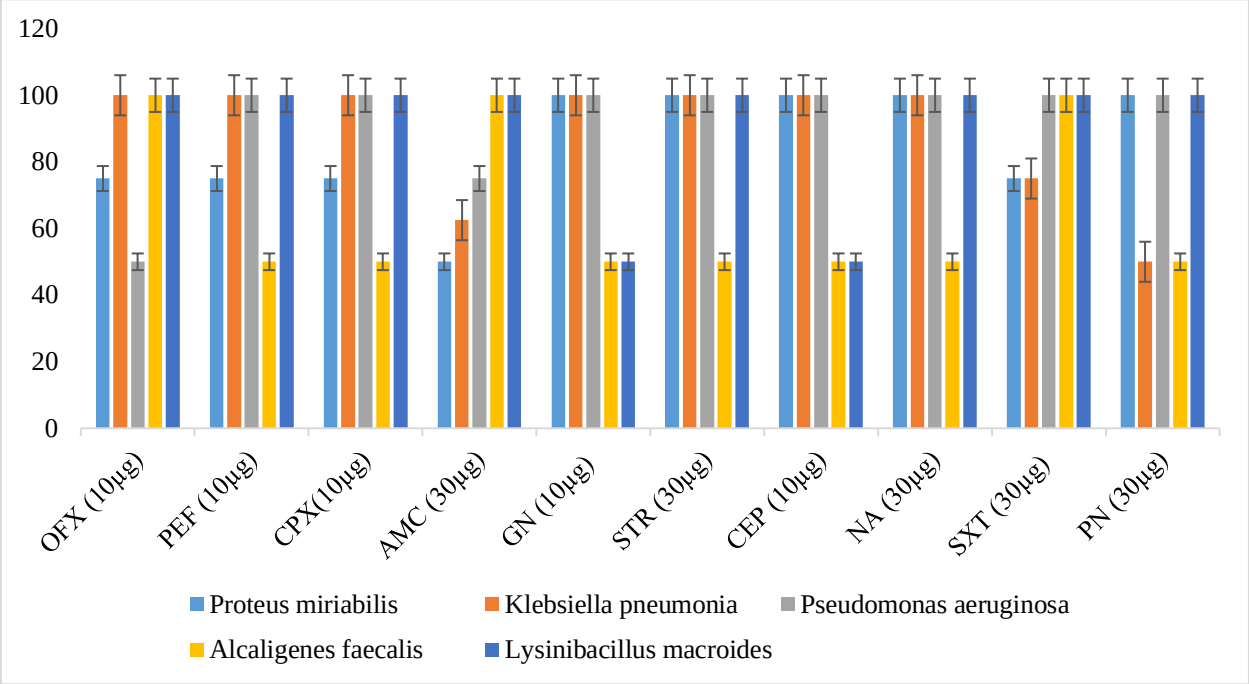

Fig. 3: Percentage of antibiotics susceptibility of selected bacteria isolates
PEF= pefloxacin, GN= gentamycin, AMC = amoxacillin clauvanic acid, CPX= ciprofloxacin, STR= streptomycin, SXT= septrin, OFX= ofloxacin, CEP = ciprorex, NA= nalidixic acid, PN = ampicillin

IV. DISCUSSION

The results of the current study have identified harmful bacteria that were isolated from the Otamiri River. There are different cases of sickness that are brought about by these germs. Pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory infection, wound infection, osteomyelitis, meningitis, bacteremia, and sepsis are among the clinical illnesses brought on by *Klebsiella pneumonia*. Typically, *Pseudomonas aeruginosa* causes blood infections as well as infections of the airways, urinary tract, burns, and wounds. Most typically, *Proteus mirabilis* is linked to urinary tract infections, particularly in difficult or infections of the urinary tract brought on by catheters. Endocarditis, bacteremia, meningitis, endophthalmitis, skin and soft tissue infections, urinary tract infections, otitis media, peritonitis, and pneumonia have all been linked to *Alcaligenes faecalis*. In people with impaired immune systems, *Lysinibacillus* can cause sepsis. Other workers have isolated and reported these bacteria throughout their exploration of the Otamiri River. *Pseudomonas, Klebsiella,* and *Proteus* have been isolated by [16][17] in their study. However, prior studies did not disclose that *Alcaligenes faecalis* and *Lysinibacillus macroides* had been identified from the Otamiri River. Bacteria are developing an ever-growing resistance to antibiotics. According to this study, the bacterium isolates tested had higher drug resistance. To the following antibiotics, *Pseudomonas aeruginosa* displayed 100% resistance: PEF, CPX, GN, STR, CEP, NA, SXT, and PN. *Pseudomonas* is one of the microorganisms isolated from water that [18, 19] reported had high antibiotic levels. 100% resistance to OFX, PEF, CPX, GN, STR, CEP, and NA was displayed by *Klebsiella pneumonia*. This concurs with the findings of [19], who noted that the bacteria showed resistance to CPX, OFX, PEF, and SXT. 100% resistance to OFX, PEF, CPX, AMC, STR, NA, SXT, and PN was demonstrated by *Lysinibacillus macroides*. The bacteria's resistance to the tested antibiotics or some of them hasn't, however, been studied. Additionally, *Pseudomonas aeruginosa* exhibited complete resistance to all antibiotics with the exception of OFX. *Pseudomonas* tested for antibiotic resistance had a 100% success rate, according to [21]. To GN, STR, CEP, NA, and PN, *Proteus mirabilis* demonstrated 100% resistance. The presence of a plasmid, the R factor, the beta lactamase enzyme, mutation, etc. may all contribute to bacterial resistance to antibiotics. Numerous genes that are resistant to antibiotics are found on mobile genetic elements, such as plasmids, transposons, and integrons. Some of these elements are easily shared across phylogenetically different bacteria. These mobile genetic elements frequently carry resistance genes for various drugs, heavy metals, and other substances [21]. The bacteria's sensitivity to the antibiotics under test revealed that they had the highest susceptibility, as demonstrated by their 50% susceptibility to OFX, PEP, CPX, AMC, GN, STR, CEP, NA, and PN. *Pseudomonas*

aeruginosa, *Klebsiella pneumonia*, and *Proteus mirabilis* isolated from rivers have all been reported to be susceptible to the antibiotics used in this study, but *Lysinibacillus macroides* and *Alcaligenes faecalis* isolates from river water have not been reported to be susceptible to any of the antibiotics

V. CONCLUSION

The results have shown that a high level of antibiotic resistance exists among the desired organisms in this investigation. Antibiotic resistance genes are present in many bacteria, which make it challenging to treat illnesses brought on by these diseases. This resistance is growing daily. The anthropogenic activities that man has engaged in his struggle for life are detrimental to all living things because of the interconnectedness of their effects. Therefore, it is necessary to preserve a peaceful environment in order to safeguard human living standards, particularly with regard to water, on which all living creatures significantly depend.

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